

BREATH TESTS FOR DISEASE AND TOXIC EXPOSURES

Michael Phillips MD, FACP

Menssana Research, Inc
and New York Medical College

A VERY BRIEF HISTORY OF BREATH TESTING

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Ancient Greece

Hippocrates - the “father of medicine”

Instructed students – smell your patient’s breath!

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Instructed students – smell your patient’s breath!

Diabetic ketoacidosis

rotten apples

Renal failure

urine-like

Liver failure

fetor hepaticus

Lung abscess

sewer-like

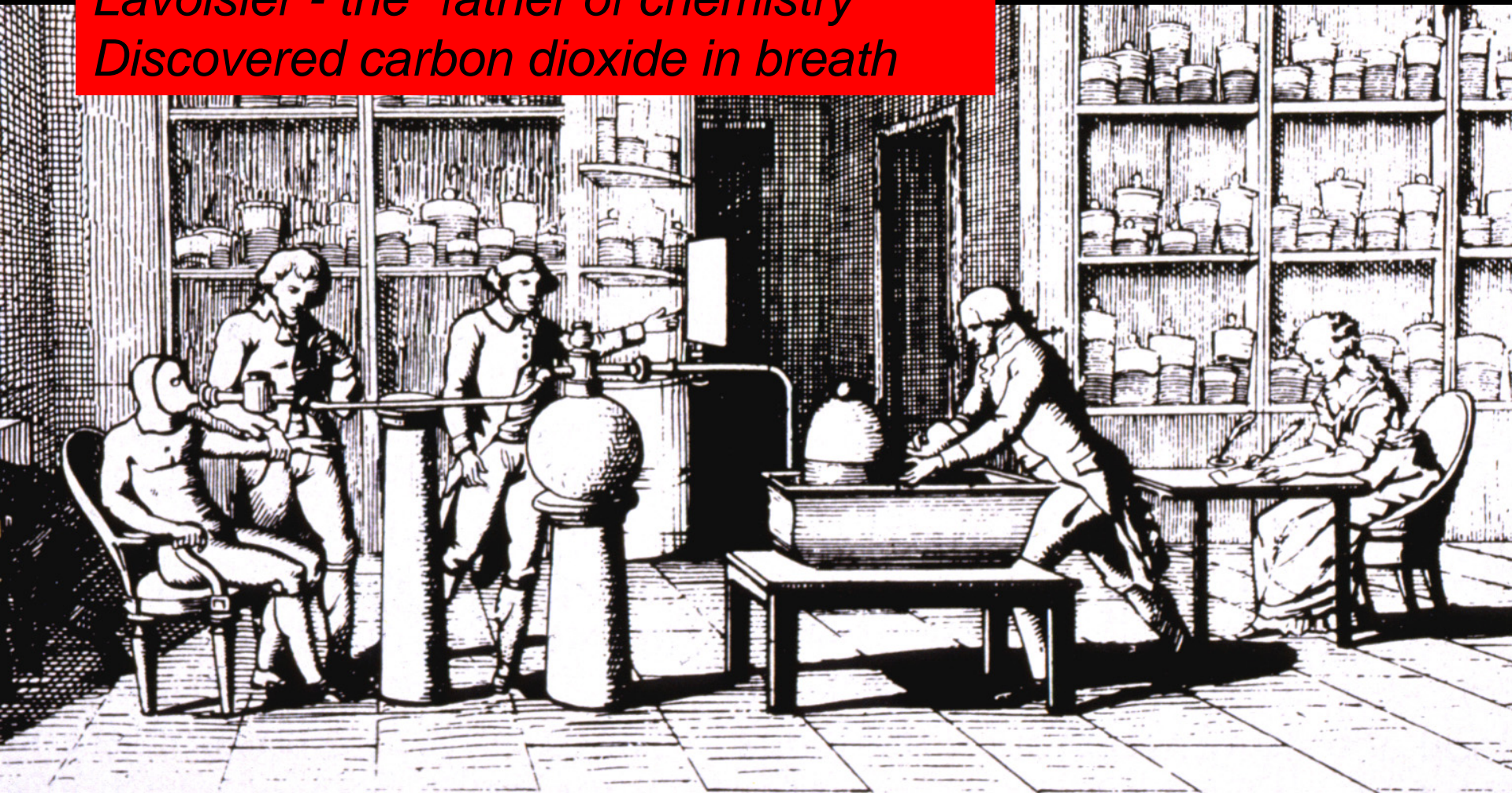
Nutrient recognition

garlic, alcohol

France – late 1700's

Lavoisier - the “father of chemistry”

Discovered carbon dioxide in breath





19th century

First colorimetric breath tests

1874 England Anstie: ethanol in drinkers

1897 Germany Nebelthau: acetone in diabetics

Little impact....

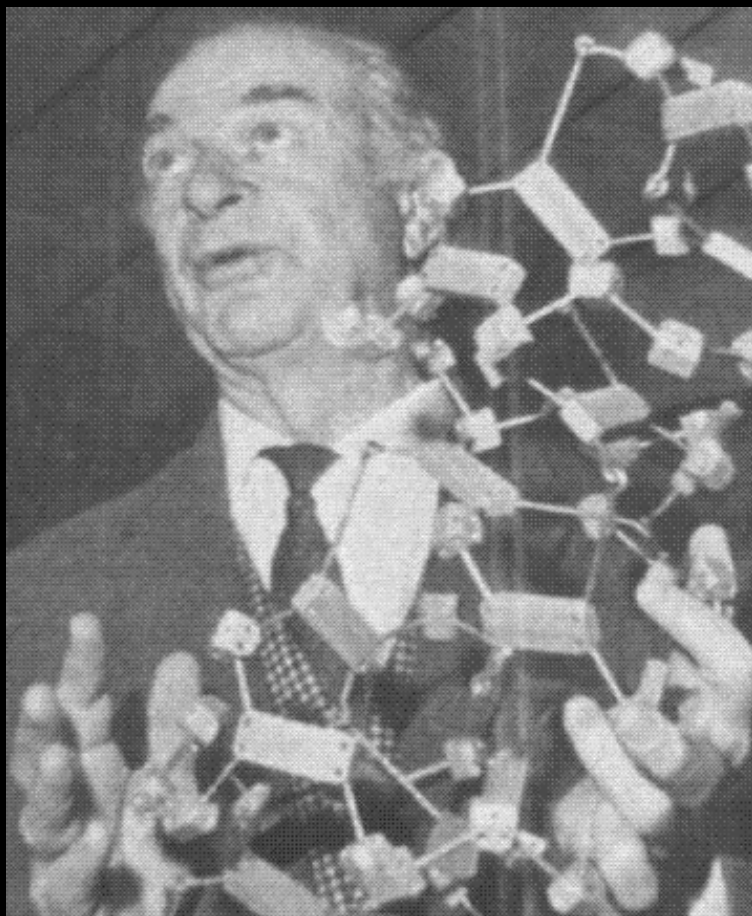
...until 1950's, in USA



Menssana Research, Inc

1960's: Breath tests in gastroenterology

- Breath hydrogen and radiolabeled CO₂
- Diagnosis of
 - malabsorption*
 - pancreatic disease*
 - liver disease*
 - bacterial overgrowth*



1971: USA

Linus Pauling

*Two Nobel Prizes
AND*

the first microanalysis of breath

Concentrated breath in a cold trap

→ GC analysis

→ discovered endogenous VOCs

Advances in the past 20 years:

- *New applications – e.g. H. pylori UBT*
- *Improved technology*
lab curiosity → clinical tool
- *Large clinical studies*
→ new biomarkers of disease

BREATH TESTING: THE FOUR QUESTIONS

HOW?

WHAT?

WHY?

WHERE?

BREATH TESTING: THE FOUR QUESTIONS

HOW do we analyze breath?

WHAT?

WHY?

WHERE?

BREATH TESTING: THE FOUR QUESTIONS

HOW do we analyze breath?

WHAT do the results mean?

WHY?

WHERE?

BREATH TESTING: THE FOUR QUESTIONS

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BREATH TESTING: THE FOUR QUESTIONS

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WHERE is it leading?

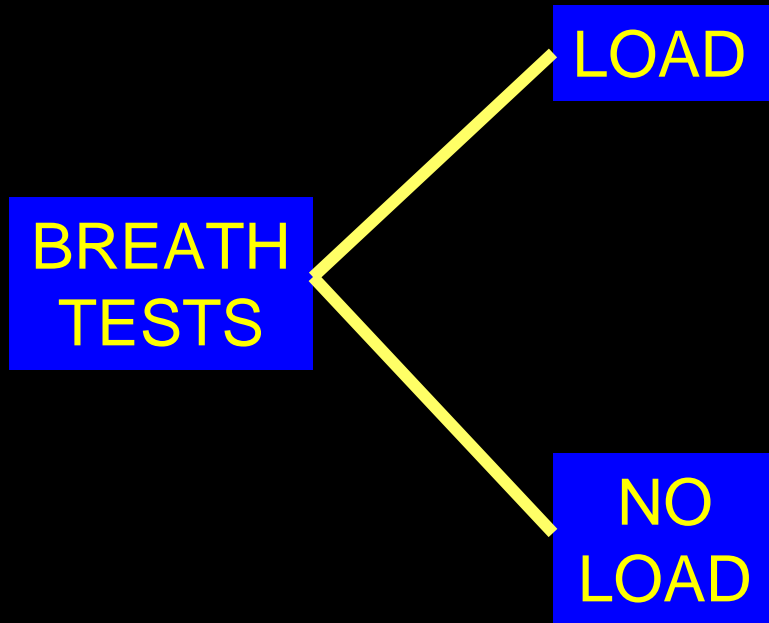
BREATH TESTING: THE FOUR QUESTIONS

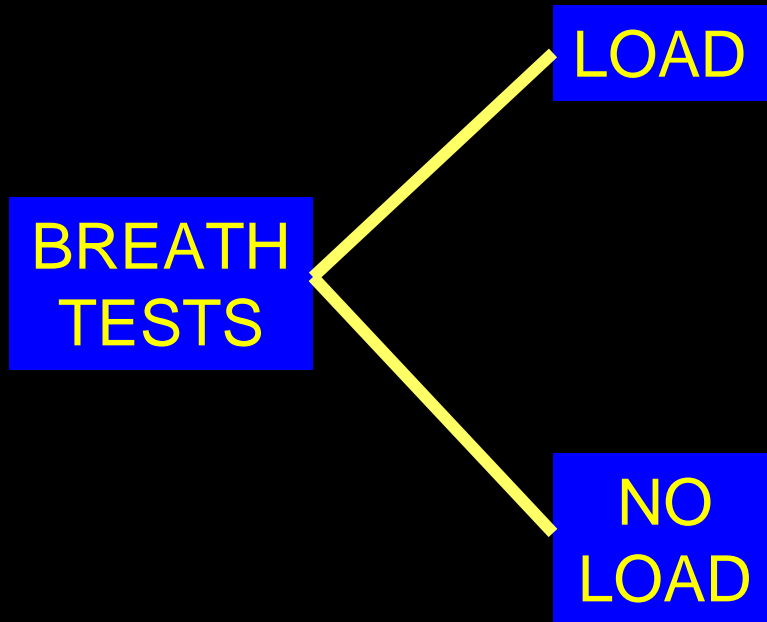
HOW do we analyze breath?

WHAT do the results mean?

WHY do it?

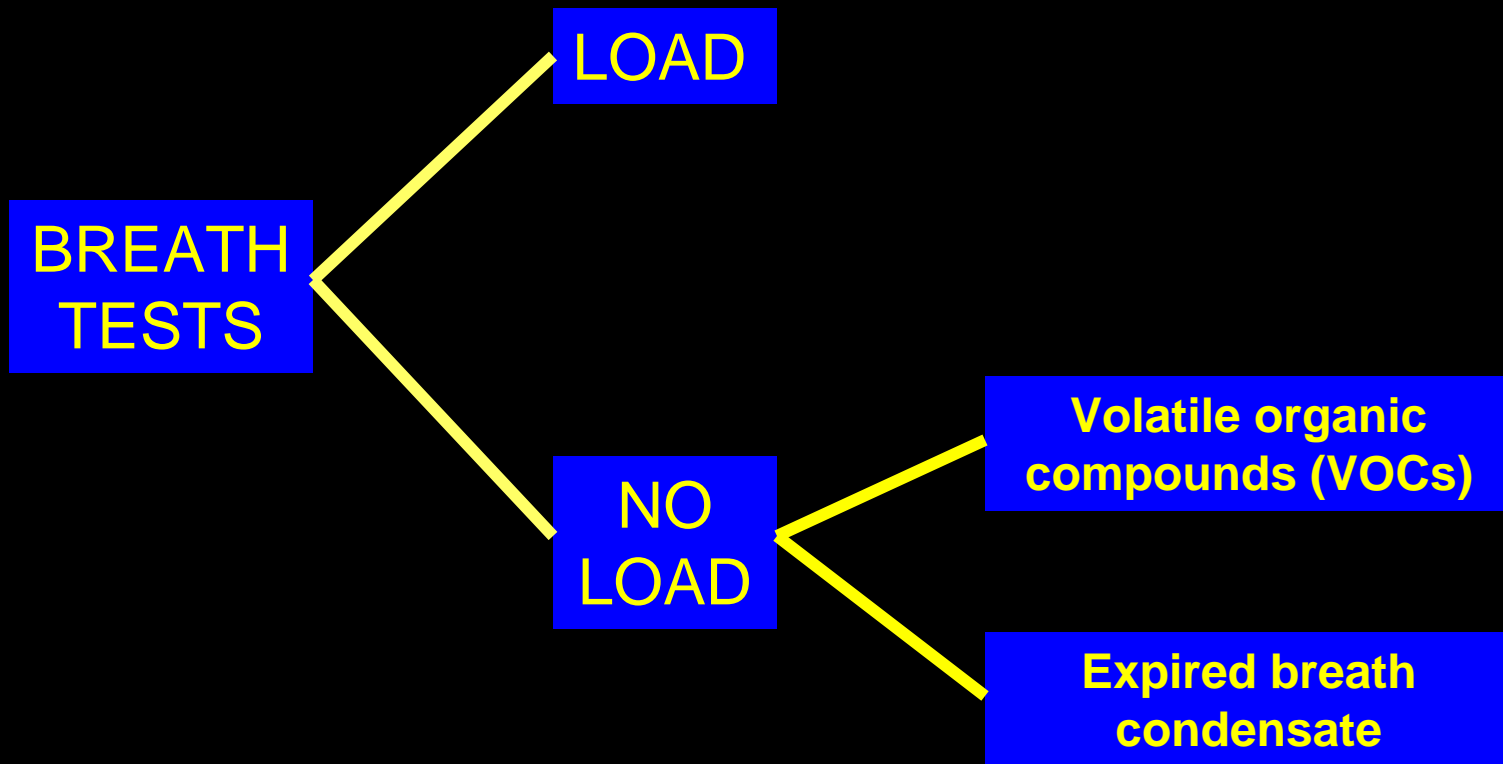
WHERE is it leading?

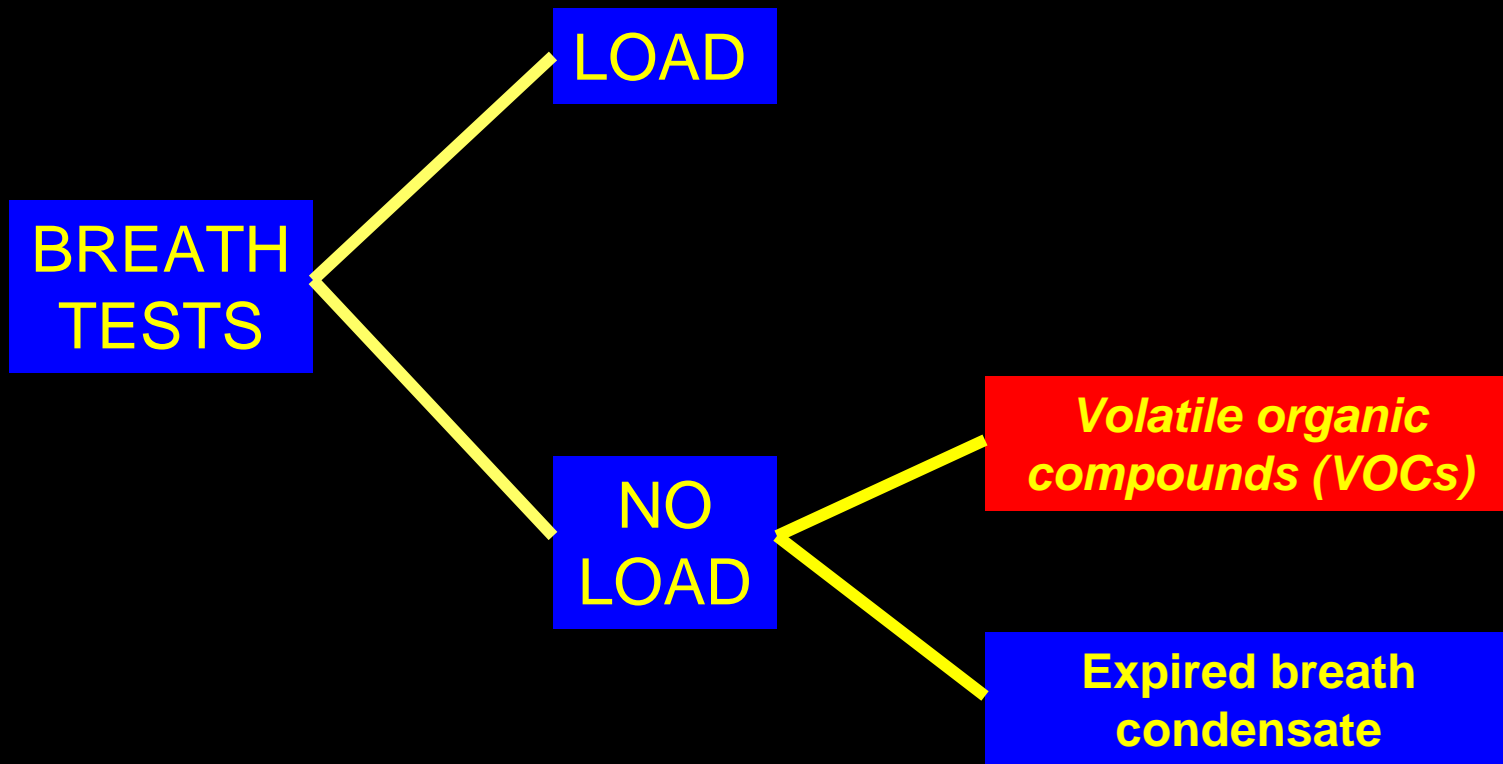


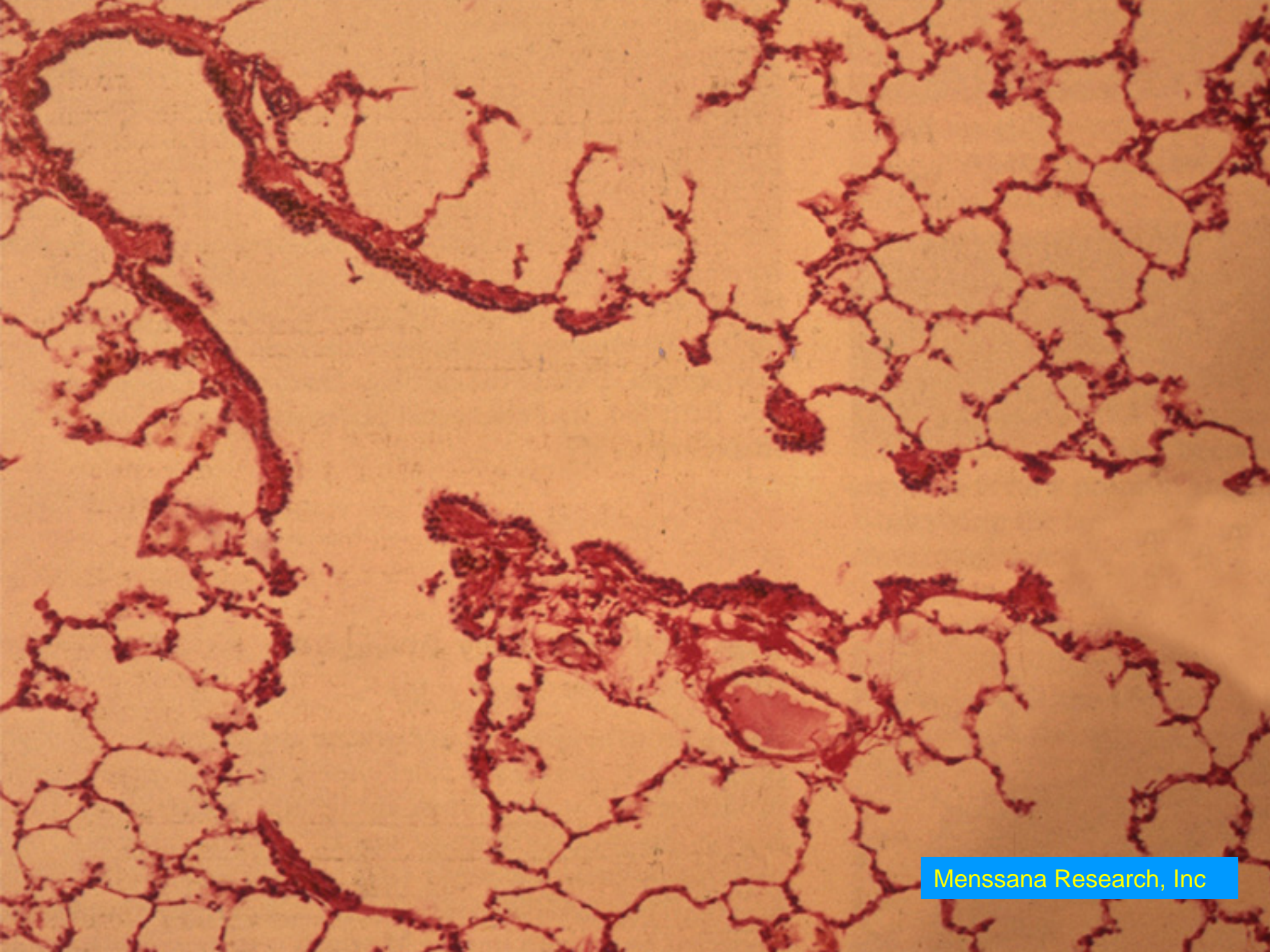


*Dose with substrate
e.g. C₁₃ labeled urea
H. pylori breath test*

*Endogenous
compounds in
breath*

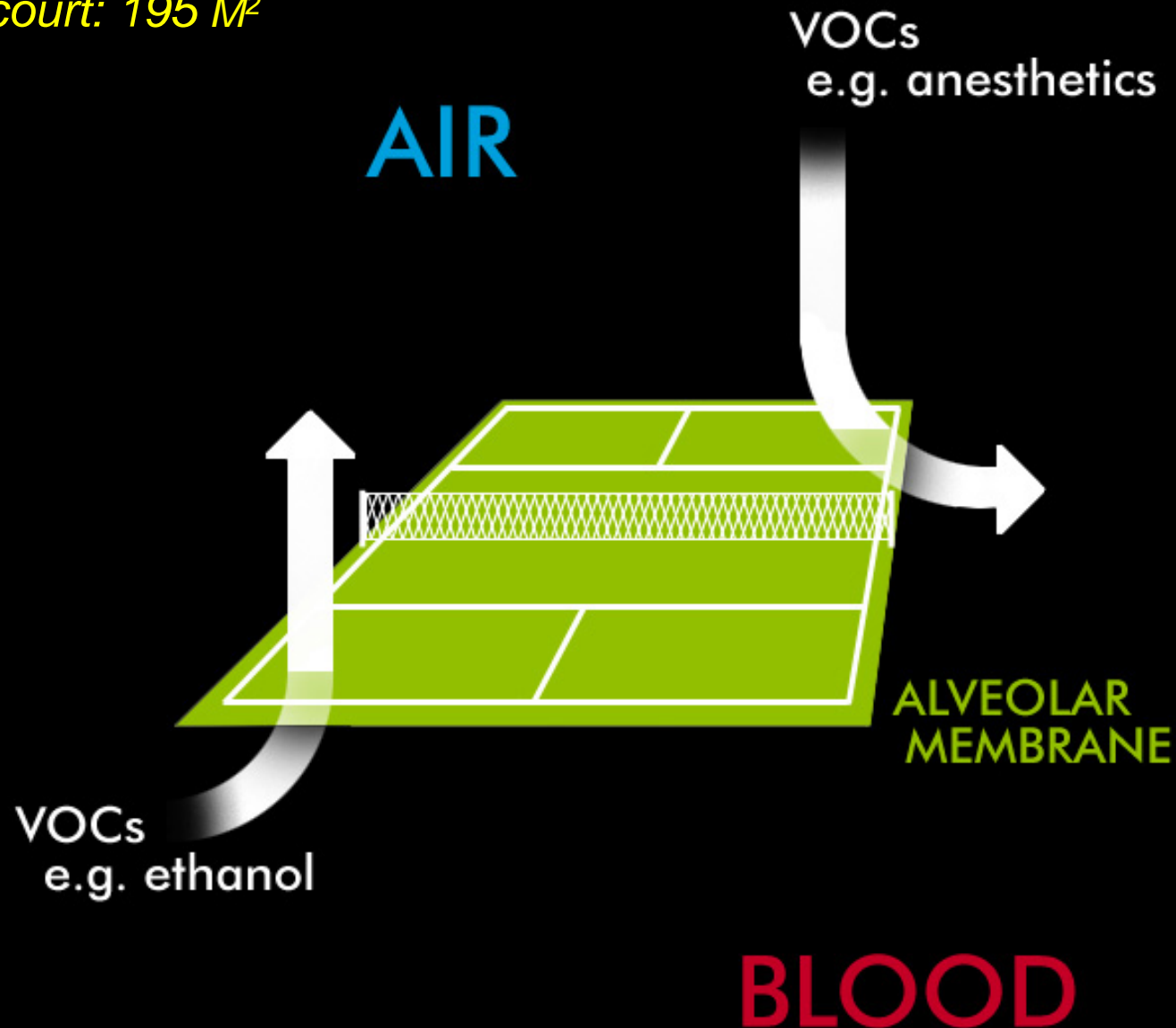






Alveolar membrane: ~120 M²

Tennis court: 195 M²



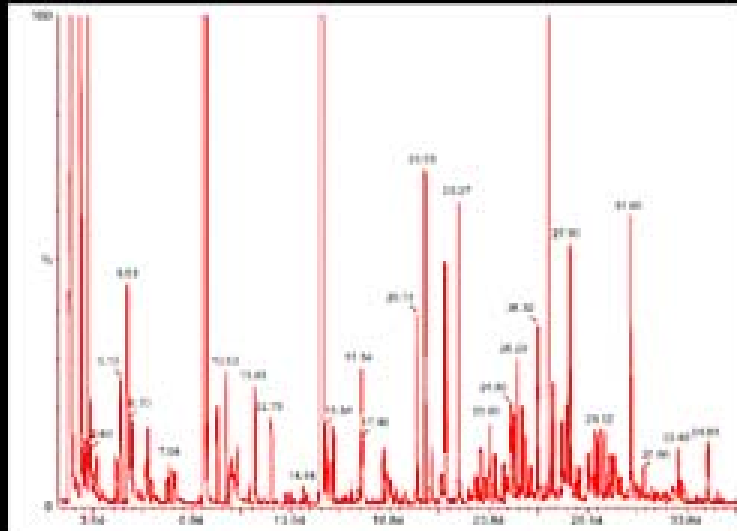


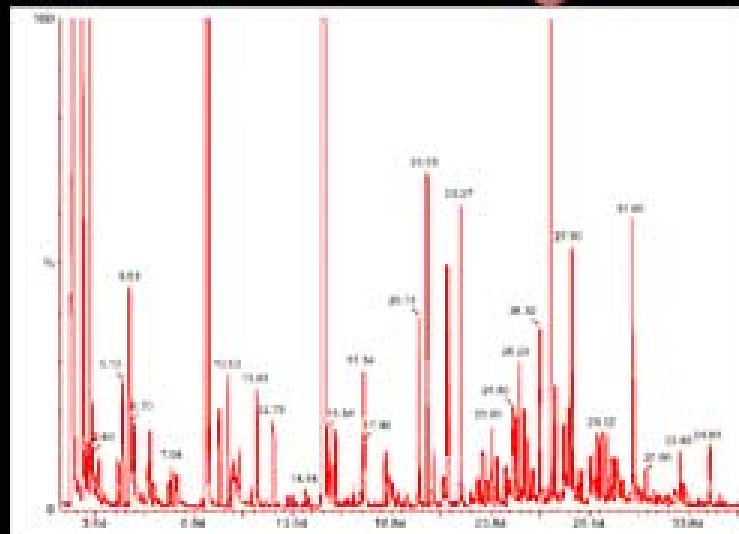
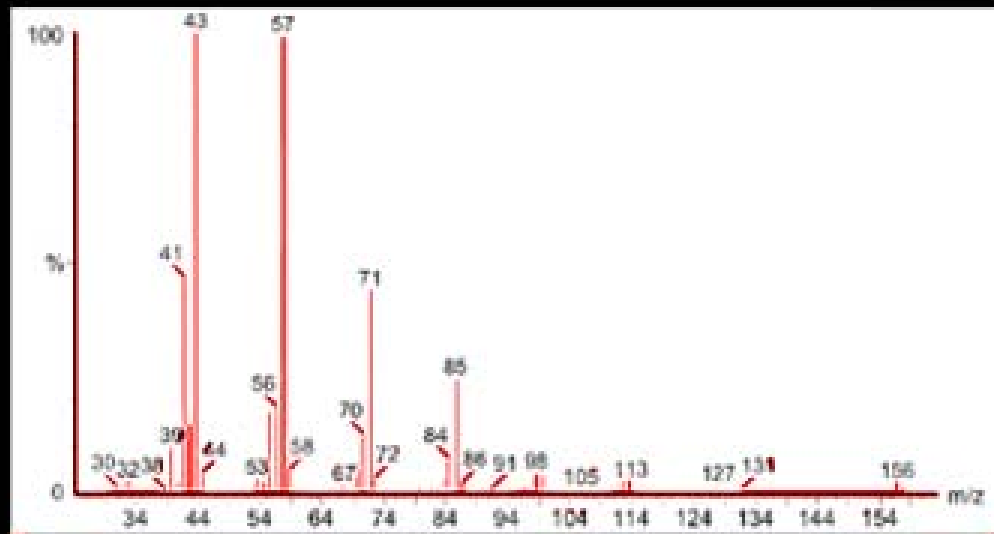
The BCA 5.0

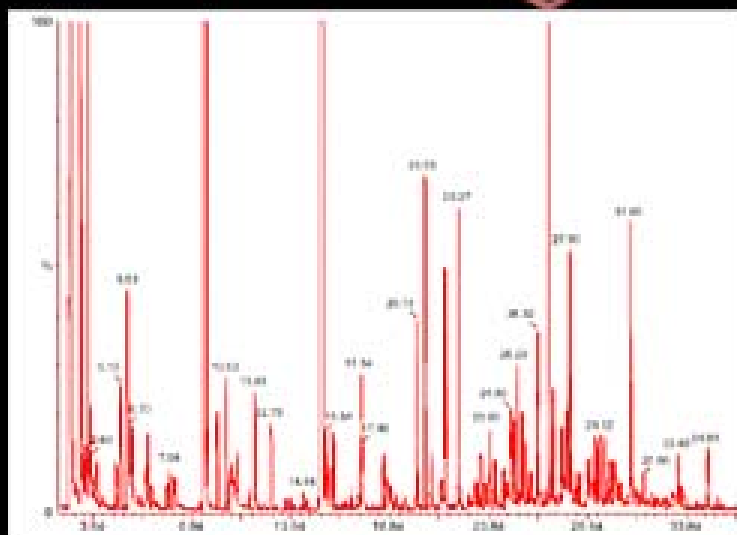
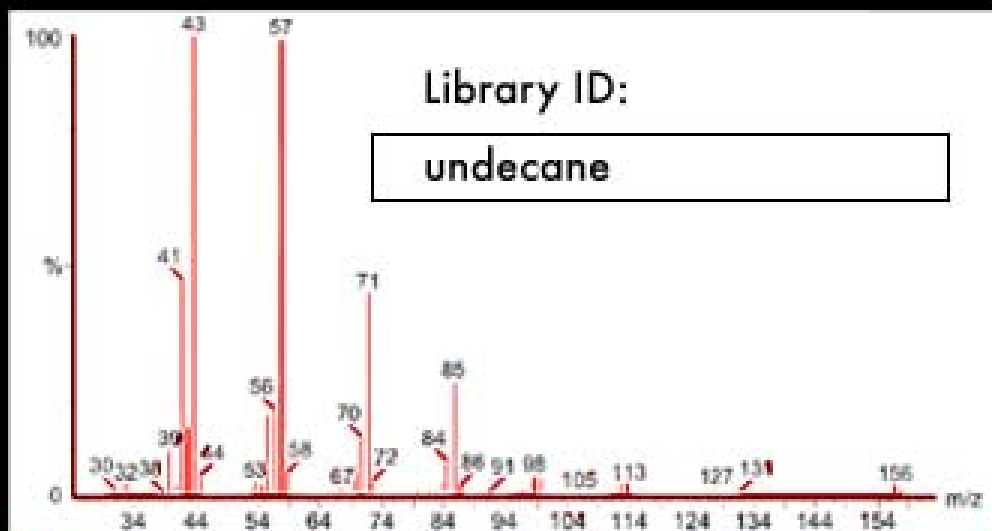
- All-electronic controls
- Digital display guides the user through every step











C:\HPCHE Norm. Hum. Study, Breath 1, Tube #173				
	RT	VOC	Area	Quality
1	1.76112	Butane	1.47E+06	49
2	2.0959	Ethanol	219710	86
3	2.19635	Butane, 2-methyl-	2.00E+06	91
4	2.39722	2-Propanone	6.18E+06	86
5	2.58135	1,3-Butadiene, 2-methyl-	2.14E+07	93
6	2.76548	1-Butene, 2-methyl-	998209	91
7	2.86592	1,2-Pentadiene	208507	86
8	2.93288	Ethane, 1,1,2-trichloro-1,2,2-trifluoro-	183187	43
9	3.08353	2,3-Pentadiene	105270	93
10	3.3681	Cyclopentene	156352	91
11	3.4518	Pentane, 3-methylene-	87692	70
12	3.56898	Butane, 2,3-dimethyl-	404068	78
13	3.71963	Pentane, 2-methyl-	1.48E+06	87
14	3.82007	Propane, 2-methoxy-2-methyl-	2.72E+06	42
15	4.12138	Pentane, 3-methyl-	1.04E+06	87
16	4.42268	1-Pentene, 2-methyl-	428950	83
17	4.75748	Hexane	998527	91
18	4.94162	Furan, 2-methyl-	683458	90
19	5.09228	2-Hexene, (E)-	270476	91
20	5.17597	2-Pentene, 2-methyl-	995042	80

192	51.4271	Octadecane	110167	90
193	51.695	2-(Methylamino)anthraquinone	41043	11
194	51.9963	Heptacosane	845932	99
195	52.4482	Octacosane	172853	91
196	52.5487	Nonadecane, 9-methyl-	207177	93
197	52.6826	AZADIBENZOPYRENE	50624	56
198	52.9169	1,2-Benzenedicarboxylic acid, bis(2-ethy	70140	68

How we analyze breath:

- *Portable breath collection apparatus*
- *Patient-friendly – no discomfort*
- *User-friendly – easy to operate*
- *Analysis with “off the shelf” instruments*
- *Identifies and quantifies breath VOCs*
- *Picomolar sensitivity (10^{-12} mol/l)*

BREATH TESTING: THE FOUR QUESTIONS

HOW do we analyze breath?

WHAT do the results mean?

WHY?

WHERE?

THE BACKGROUND AIR PROBLEM

Improved technology revealed:

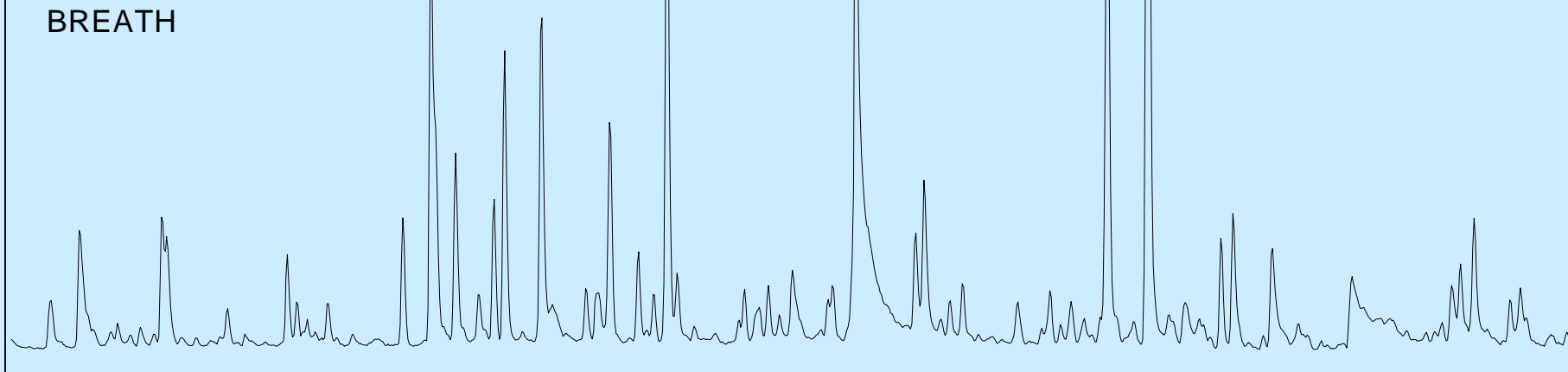
Most breath VOCs are also present in room air!

Are breath VOCs recycled room air VOCs?

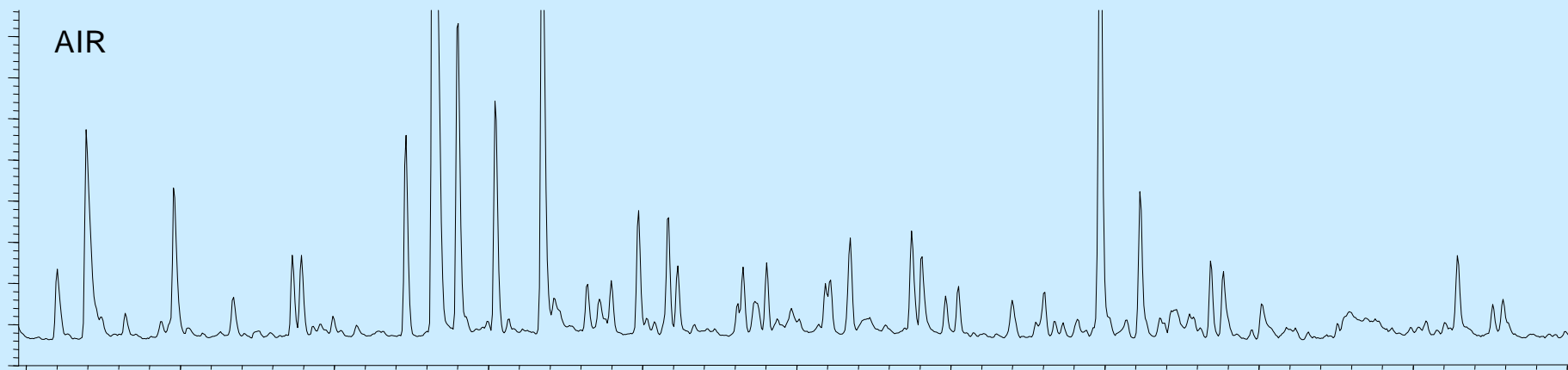
Options

1. Ignore the problem
2. Supply patient with ultra-pure air
3. Subtract air background from breath

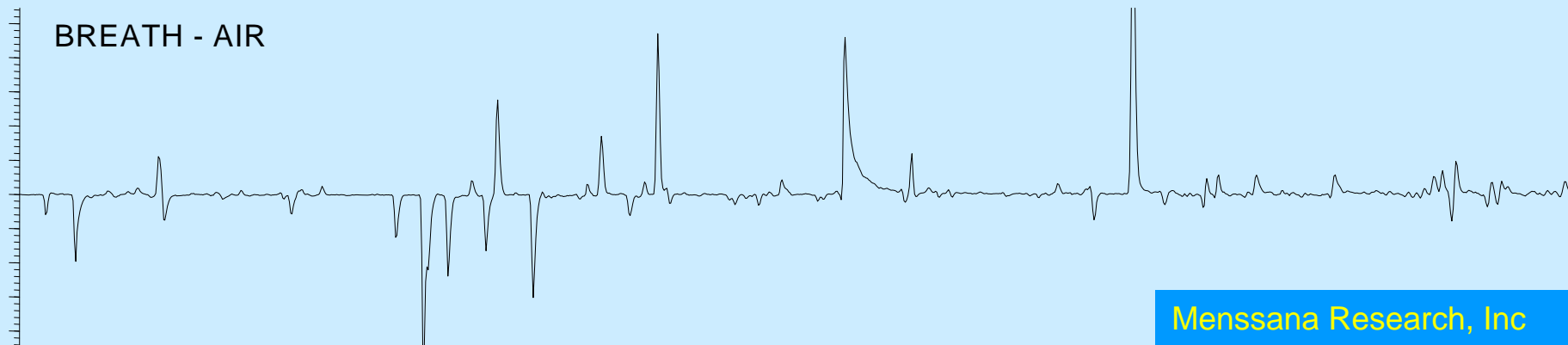
BREATH



AIR



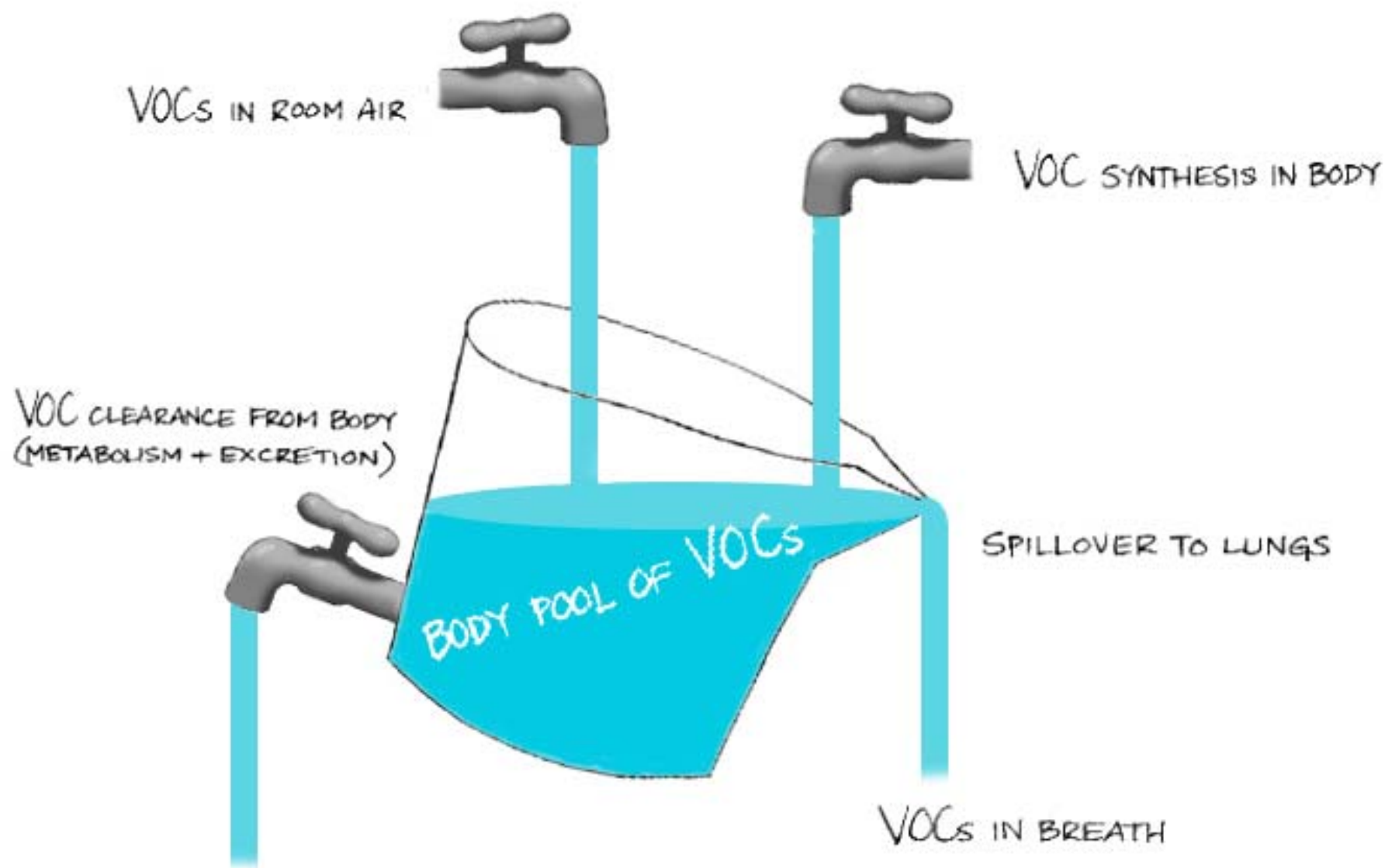
BREATH - AIR

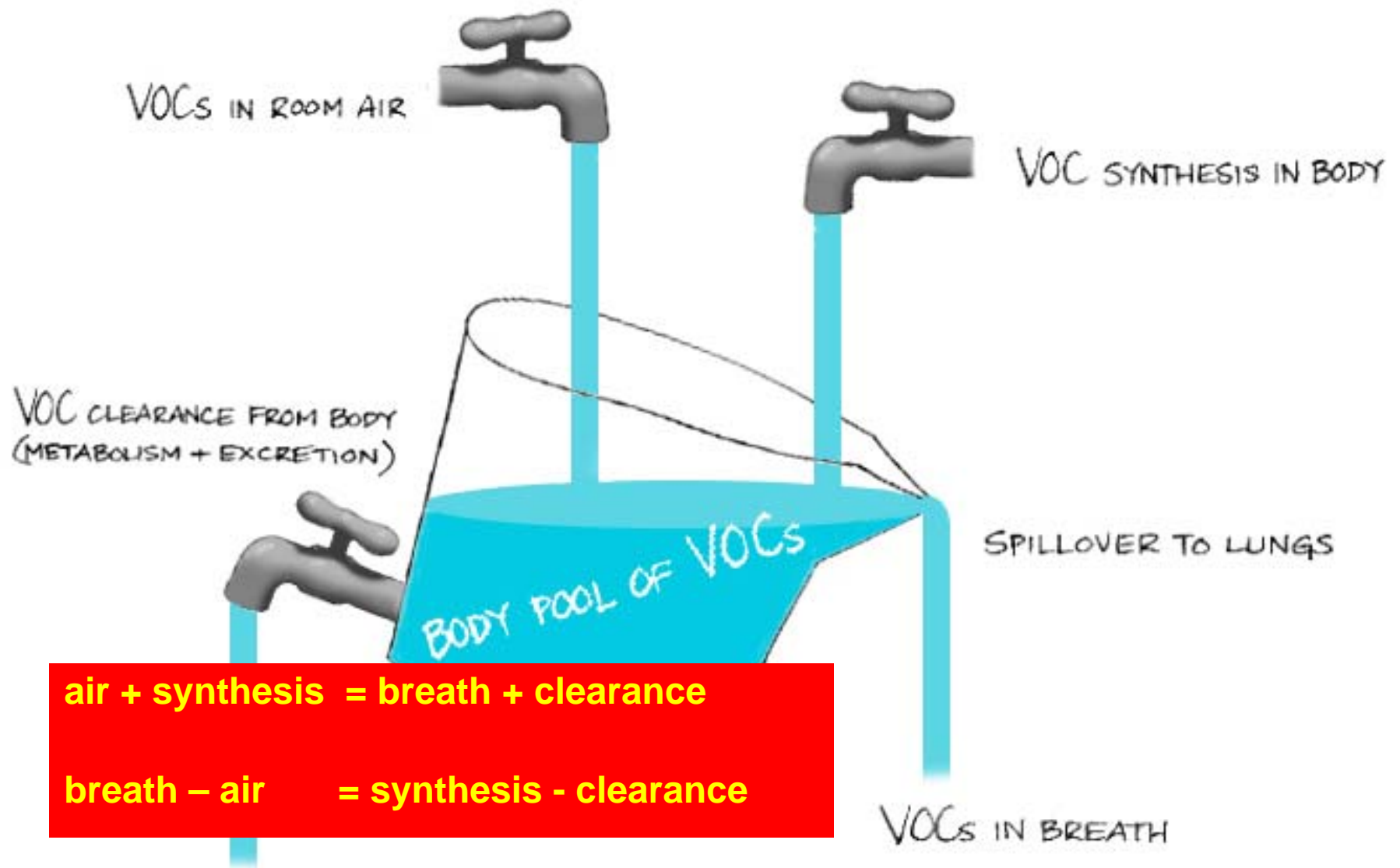


THE ALVEOLAR GRADIENT

$$= \text{Concentration}_{\text{breath}} - \text{Concentration}_{\text{room air}}$$

$$\text{varies with: } \text{Rate}_{\text{synthesis}} - \text{Rate}_{\text{clearance}}$$





WHAT ARE THE VOCs IN NORMAL HUMAN BREATH?

Study

Breath tests in 50 fasting normal humans

Results

VOCs in each person	app. 200
Total different VOCs	3481
Positive alveolar gradient	1753
Negative alveolar gradient	1728

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PROBLEM! TOO MUCH DATA!

WHAT ARE THE VOCs IN NORMAL HUMAN BREATH?

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Results

VOCs in each person	app. 200
Total different VOCs	3481
Positive alveolar gradient	1753
Negative alveolar gradient	1728
Common core VOCs	27

COMMON CORE VOCs

GRADIENT POSITIVE

isoprene

benzene, 1-methylethenyl

naphthalene

cyclohexadiene

naphthalene, 1-methyl

butane, 2-methyl

tetradecane

pentadecane

dodecane

GRADIENT NEGATIVE

benzene

benzene, 1-ethyl-2-methyl

benzene, ethyl

benzene, methyl

benzene, propyl

cyclohexane, methyl

decane

heptane

heptane, 2-methyl

heptane, 3-methyl

hexane

hexane, 3-methyl

nonane

pentane, 2,3,4-trimethyl

pentane, 2-methyl

pentane, 3-methyl

propane, 2-methoxy-2-methyl

undecane

COMMON CORE VOCs

GRADIENT POSITIVE

isoprene

benzene, 1-methylethenyl

naphthalene

cyclohexadiene

naphthalene, 1-methyl

butane, 2-methyl

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dodecane

GRADIENT NEGATIVE

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benzene, ethyl

benzene, methyl

benzene, propyl

cyclohexane, methyl

decane

heptane

heptane, 2-methyl

heptane, 3-methyl

hexane

hexane, 3-methyl

nonane

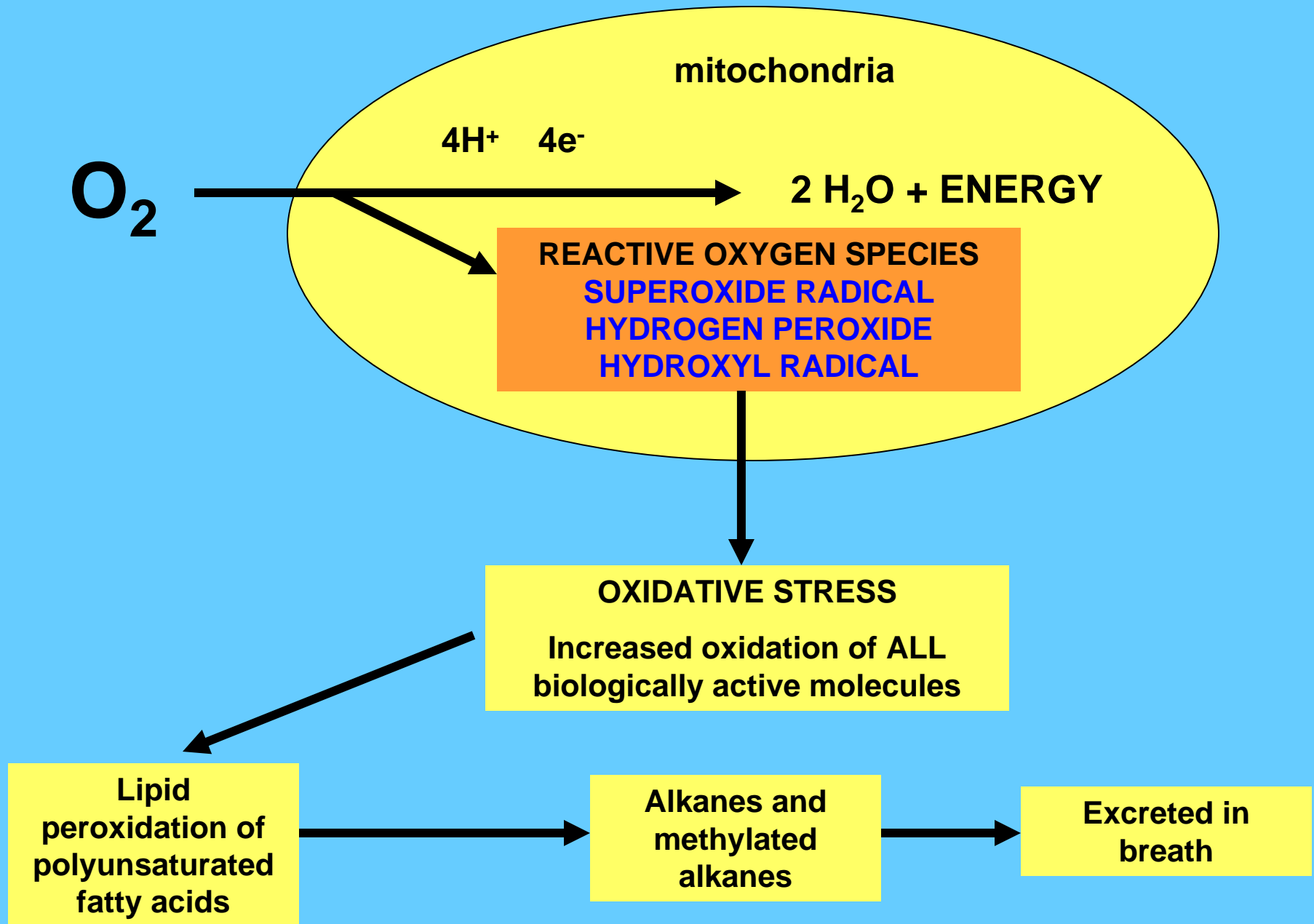
pentane, 2,3,4-trimethyl

pentane, 2-methyl

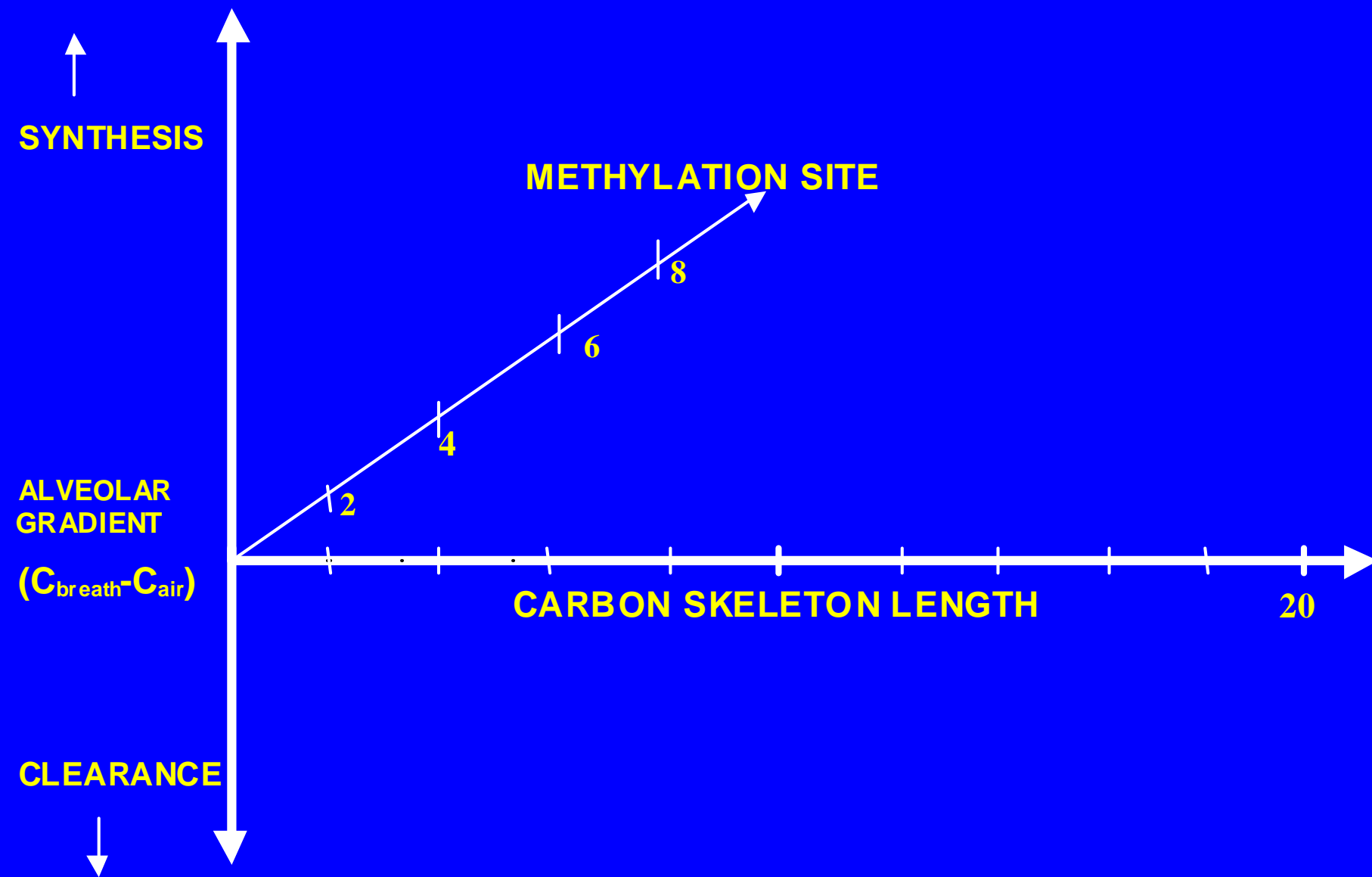
pentane, 3-methyl

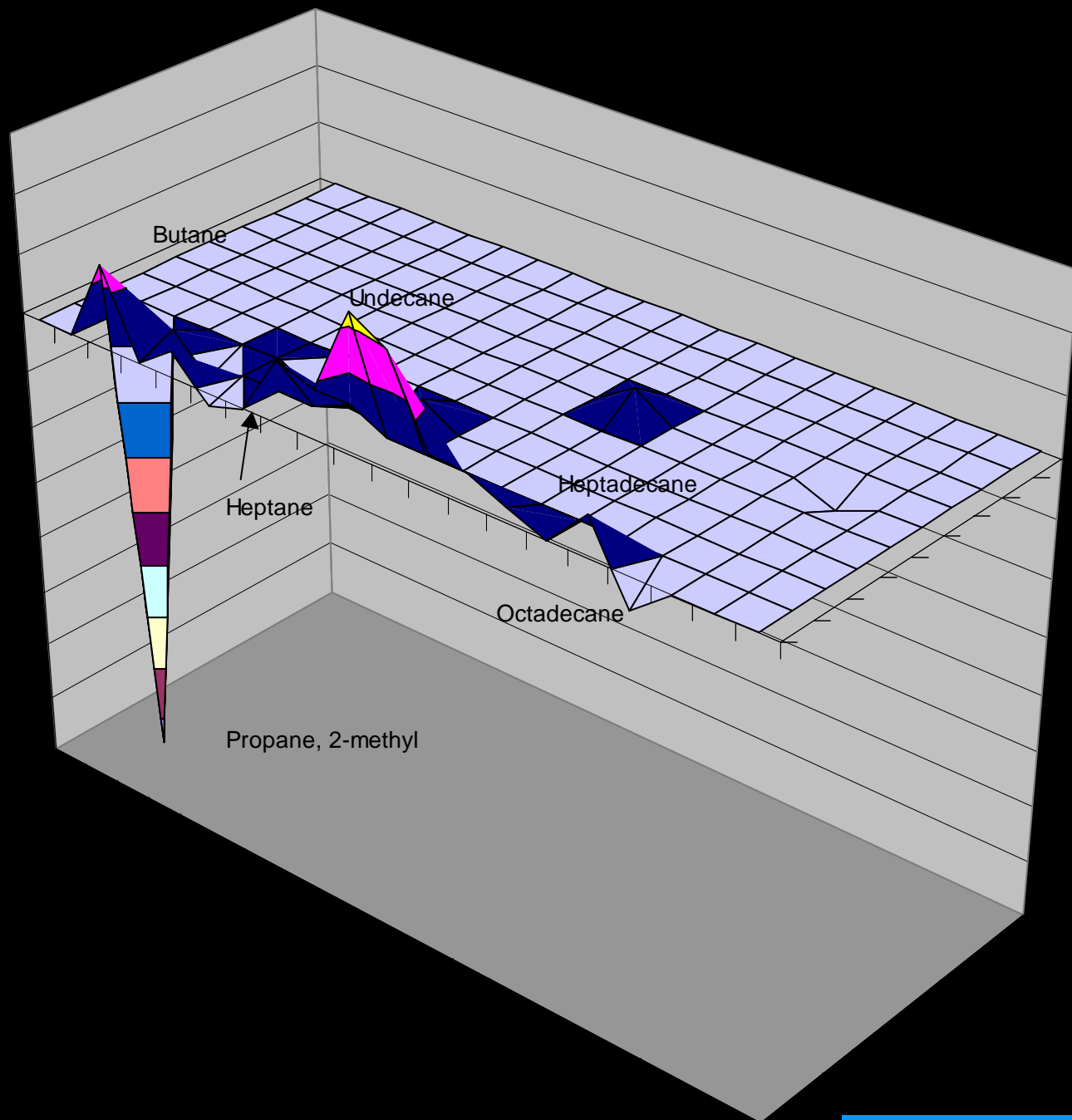
propane, 2-methoxy-2-methyl

undecane



BREATH METHYLATED ALKANE CONTOUR





WHAT THE RESULTS MEAN

- VOCs are present in breath and room air
→ essential to measure both!
- Everyone has VOC markers of oxidative stress in their breath
- BMAC: displays >100 oxidative stress markers

BREATH TESTING: THE FOUR QUESTIONS

HOW do we analyze breath?

WHAT do the results mean?

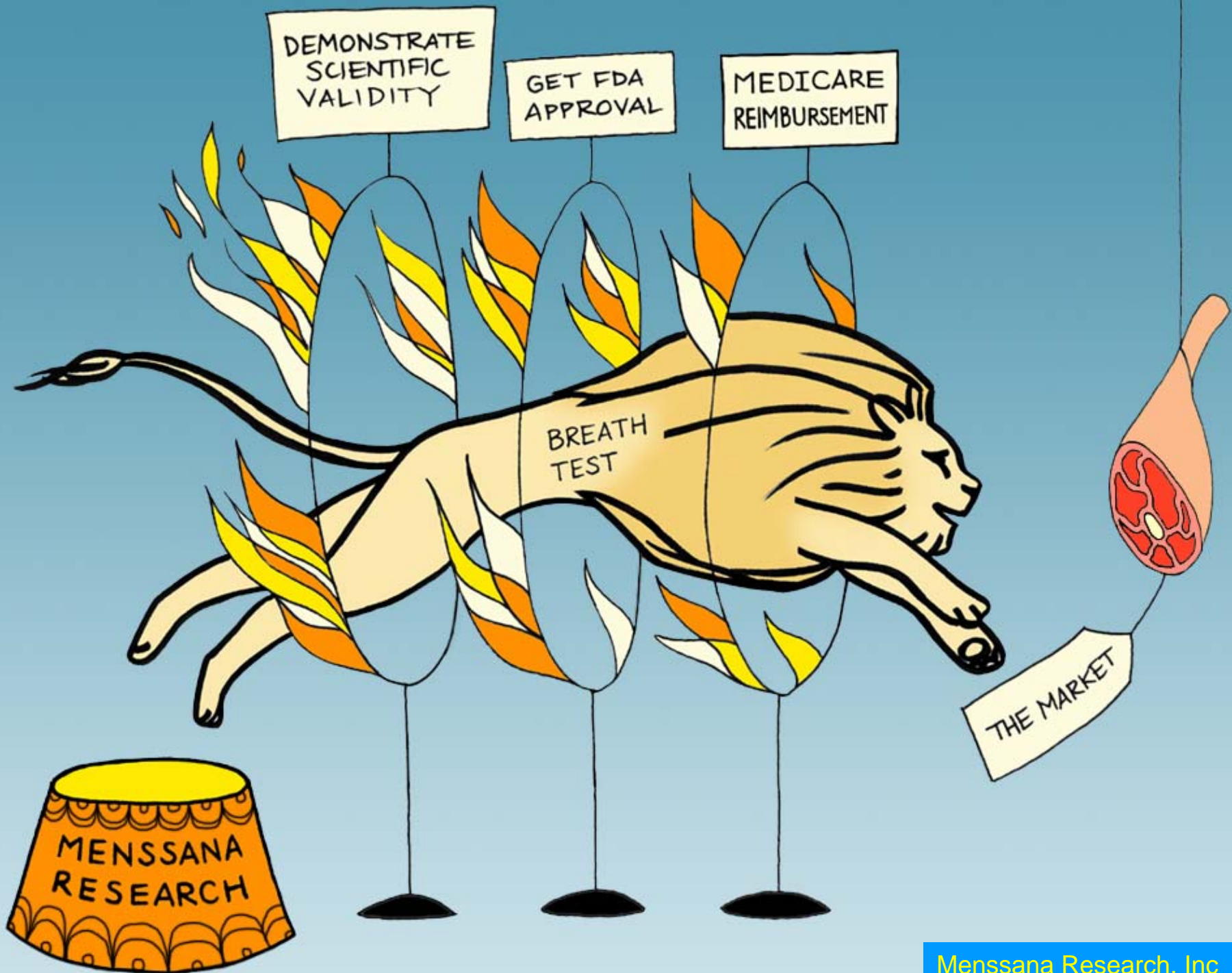
WHY do it?

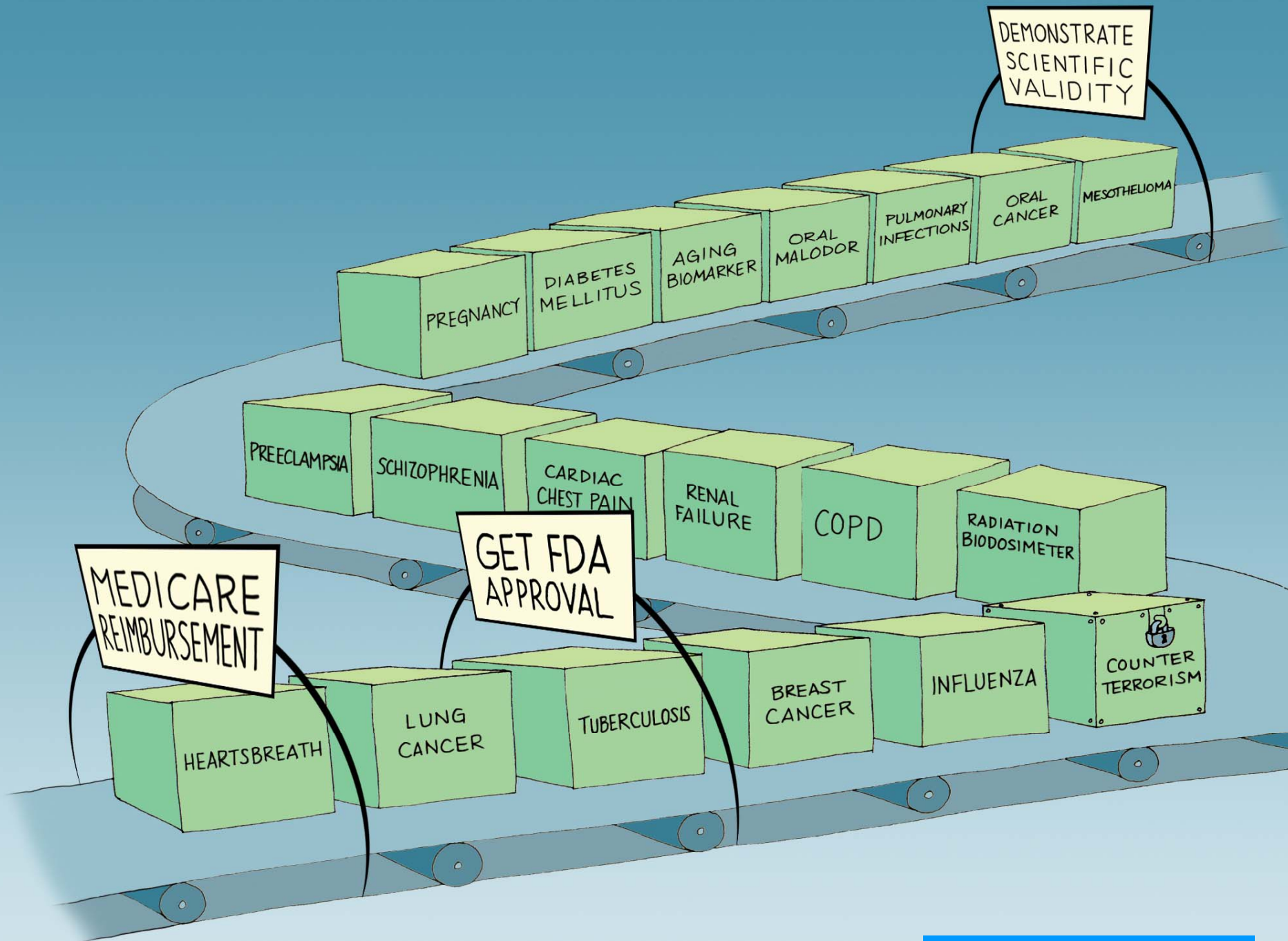
WHERE is it leading?

BREATH TESTS

Disease markers for the 21st century:

- *Accurate*
- *Non-invasive*
- *Cost-effective*
- *Safe*





THE HEARTSBREATH TEST FOR HEART TRANSPLANT REJECTION

The problem

- ~2,500 heart transplants a year – most in USA
- High risk of rejection in first year
- Patients monitored with ~20 heart biopsies
 - *Grade 0, 1 or 2 versus grade 3*
 - *observe versus treat*
- Painful, invasive, poor accuracy
- *Expensive!* ~ \$3,500 (Medicare → Aetna etc)

Flaming hoop #1: Establish scientific validity

- NIH/NHLBI Phase I and Phase II SBIR awards
- Heart Allograft Rejection: Detection with Breath Alkanes in Low Levels (the HARDBALL study)
- Seven academic medical centers
- >1,100 patient studies

*→ Heartsbreath test:
sensitive and specific
for Grade 3 rejection*



Flaming hoop #2: Get FDA approval



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[How We Can Help](#)

[Resources](#)

[Problems with Medical Devices](#)

New Humanitarian Device Approval

Heartsbreath - H030004

FDA approved this device under the [Humanitarian Device Exemption \(HDE\)](#) program. See the links below to the [Summary of Safety and Probable Benefit \(SSPB\)](#) and other sites for more complete information on this product, its indications for use, and the basis for FDA's approval.

Product Name: Heartsbreath

Manufacturer: Menssana Research, Inc.

Address: 1 Horizon Road, Suite 1415, Fort Lee, NJ 07024-6510

Approval Date: February 24, 2004

Approval Letter: <http://www.fda.gov/cdrh/ode/H030004sum.html>

We want you to know™



***Flaming hoop #3:
Get insurance to
pay for it***

...Aetna considers the
HeartsbreathTest (Menssana
Research, Inc., Fort Lee, NJ)
medically necessary for use as
an aid to diagnosis of grade 3
heart transplant rejection in
persons who have received
heart transplants within the
preceding year.

We want you to know™



...Aetna considers the
HeartsbreathTest (Menssana
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an aid to diagnosis of grade 3
heart transplant rejection in
persons who have received
heart transplants within the
preceding year.



NCA Tracking Sheet for Heartsbreath Test for Heart Transplant Rejection (CAG-00394N)

- *National Coverage Determination – in progress*

BREATH TEST FOR LUNG CANCER

3 multicenter clinical studies

Lancet 1998

Chest 2003

Cancer Biomarkers 2007

Similar findings in all studies

- Breath test sensitive and specific for lung cancer
- TNM stage → NO effect on accuracy of breath test
- Potential application:
- Early detection Stage 1 lung cancer
→ improve survival?

CONCLUSIONS FROM THIRD CLINICAL STUDY

- Breath test predicts lung cancer
- Predicts Stage 1 before metastasis
- Sensitivity and specificity similar to chest CT
- Predictions not affected by smoking
- Cost effective, safe screening test
 - early detection?
 - save lives?



Volatile biomarkers of pulmonary tuberculosis in the breath

Michael Phillips^{a,b,*}, Renee N. Cataneo^a, Rany Condos^c, Gerry Ring Erickson^d, Joel Greenberg^{a,✉}, Vincent La Bombardi^e, Muhammad I. Munawar^a, Olaf Tietje^f

^a*Menssana Research Inc., Fort Lee, NJ 07024, USA*

^b*Department of Medicine, New York Medical College, Valhalla, NY, USA*

^c*Division of Pulmonary and Critical Care Medicine, Bellevue Chest Service, NYU School of Medicine, New York, NY, USA*

^d*Infometrix, Inc, Woodinville, WA, USA*

^e*Saint Vincent's Medical Center, New York, NY, USA*

^f*SystAim GmbH, Pfingstweidstr. 31a, CH 8005 Zürich, Switzerland*

Received 14 December 2005; received in revised form 8 March 2006; accepted 10 March 2006

How can a breath test identify patients with active pulmonary TB?

How can a breath test identify patients with active pulmonary TB?

...Because VOC biomarkers in breath are the same or similar to VOC metabolites of Mycobacteria in vitro!

VOC biomarkers of Mycobacteria

Culture (in vitro)		
Naphthalene, 1-methyl-		
3-Heptanone		
Methylcyclododecane		
Heptane, 2,2,4,6,6-pentamethyl-		
Benzene, 1-methyl-4-(1-methylethyl)-		
Cyclohexane, 1,4-dimethyl-		
3,5-dimethylamphetamine		
Butanal, 3-methyl-		
2-Hexene		
Trans-anti-1-methyl-decahydronaphthalene		

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Naphthalene, 1-methyl-		
3-Heptanone		
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Cyclohexane, 1,4-dimethyl-		
3,5-dimethylamphetamine		
Butanal, 3-methyl-		
2-Hexene		
Trans-anti-1-methyl-decahydronaphthalene		

Breath (fuzzy logic)		
Cyclohexane, 1,3-dimethyl-, trans-		
Benzene, 1,4-dichloro-		
Cyclohexane, 1,4-dimethyl-		
1-Octanol, 2-butyl-		
2-Butanone		
Naphthalene, 1-methyl-		
Camphene		
Decane, 4-methyl-		
Heptane, 3-ethyl-2-methyl-		
Octane, 2,6-dimethyl-		
Benzene, 1,2,3,4-tetramethyl-		
Bicyclo_3_1_1_hept-2-ene, 3,6,6-trimethyl-		
Cyclohexane, 1-ethyl-4-methyl-, trans-		
I-_beta_-Pinene		

IDENTICAL VOCs

Culture (in vitro)

Naphthalene, 1-methyl-

3-Heptanone

Methylcyclododecane

Heptane, 2,2,4,6,6-pentamethyl-

Benzene, 1-methyl-4-(1-methylethyl)-

Cyclohexane, 1,4-dimethyl-

3,5-dimethylamphetamine

Butanal, 3-methyl-

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Breath (fuzzy logic)

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Benzene, 1,4-dichloro-

Cyclohexane, 1,4-dimethyl-

1-Octanol, 2-butyl-

2-Butanone

Naphthalene, 1-methyl-

Camphene

Decane, 4-methyl-

Heptane, 3-ethyl-2-methyl-

Octane, 2,6-dimethyl-

Benzene, 1,2,3,4-tetramethyl-

Bicyclo_3_1_1_hept-2-ene, 3,6,6-trimethyl-

Cyclohexane, 1-ethyl-4-methyl-, trans-

I_beta_Pinene

IDENTICAL AND SIMILAR VOCs

Culture (in vitro)

Naphthalene, 1-methyl-

3-Heptanone

Methylcyclododecane

Heptane, 2,2,4,6,6-pentamethyl-

Benzene, 1-methyl-4-(1-methylethyl)-

Cyclohexane, 1,4-dimethyl-

3,5-dimethylamphetamine

Butanal, 3-methyl-

2-Hexene

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Breath (fuzzy logic)

Cyclohexane, 1,3-dimethyl-, trans-

Benzene, 1,4-dichloro-

Cyclohexane, 1,4-dimethyl-

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2-Butanone

Naphthalene, 1-methyl-

Camphene

Decane, 4-methyl-

Heptane, 3-ethyl-2-methyl-

Octane, 2,6-dimethyl-

Benzene, 1,2,3,4-tetramethyl-

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Decane, 4-methyl-
Heptane, 3-ethyl-2-methyl-
Octane, 2,6-dimethyl-
Benzene, 1,2,3,4-tetramethyl-
Bicyclo_3_1_1_hept-2-ene, 3,6,6-trimethyl-
Cyclohexane, 1-ethyl-4-methyl-, trans-
l_beta_Pinene

VOC manufactured
by Mycobacteria



Excreted in breath:
unchanged or a
metabolite

BREATH TESTING: THE FOUR QUESTIONS

HOW do we analyze breath?

WHAT do the results mean?

WHY do it?

WHERE is it leading?

Lab Test



```
graph TD; A[Lab Test] --> B[Office test]; B --> C[End-user test];
```

Office test

End-user test

*Evolution of a
diagnostic test*

Pregnancy tests

Lab Test



1927 *Ascheim Zondek test*
\$\$\$ *Market +*

Office test



1960 *Immunological tests*
\$\$ *Market ++*

End-user test

1976
First home test kit
~\$10 *Market ++++*

Lab Test

```
graph TD; A[Lab Test] --> B[Office test]; B --> C[End-user test];
```

*Basic R&D (GC/MS)
→ develop algorithms*

Office test

*Apply algorithms in
office instrument:
→cheaper, quicker*

End-user test

*Shrink the instrument:
→simple, cheap, quick*

Lab Test

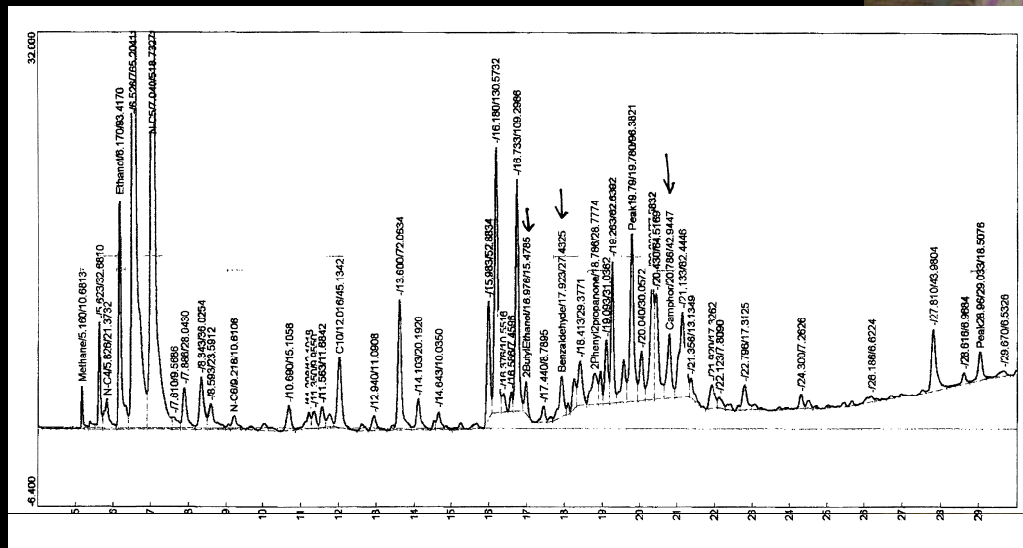
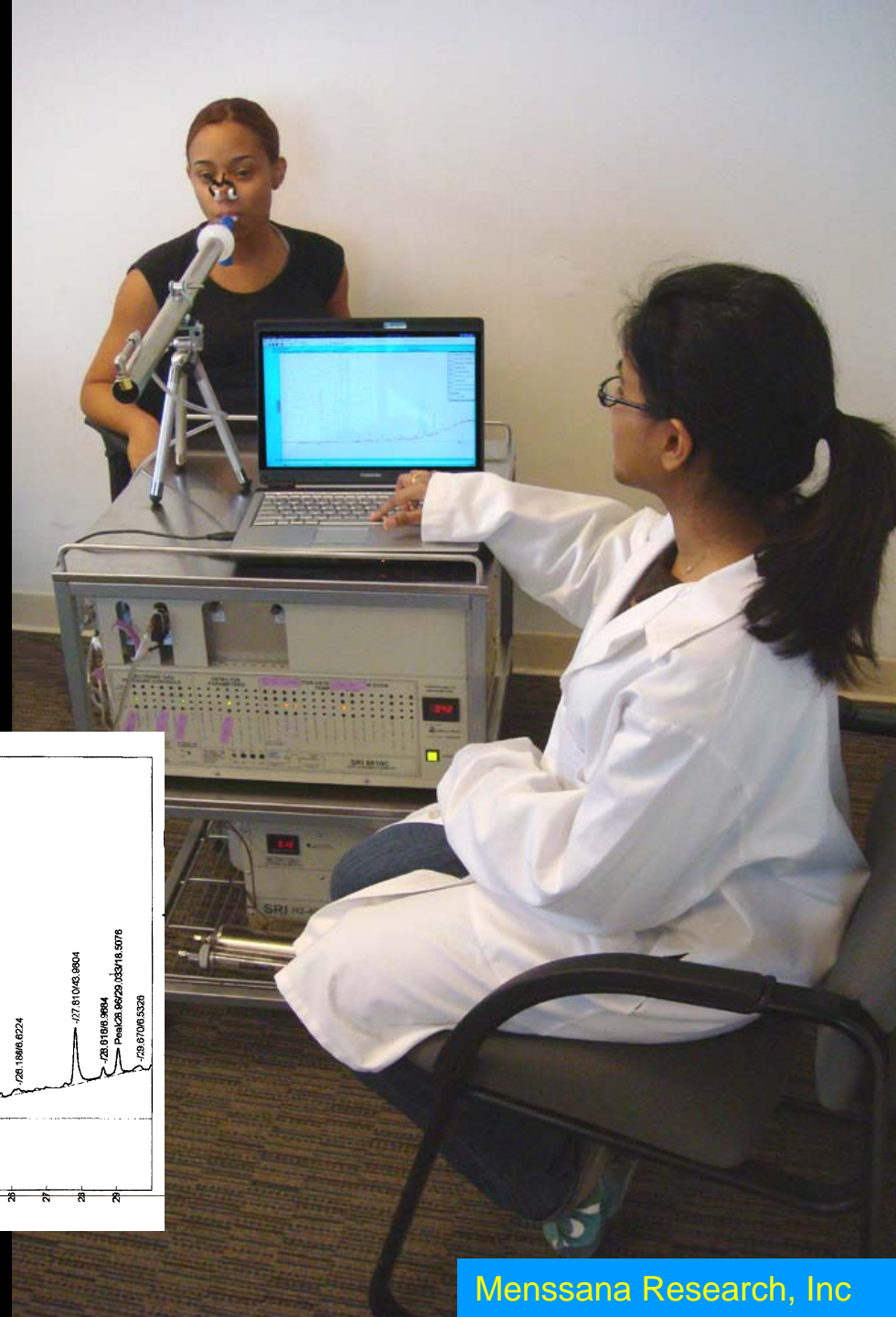
```
graph TD; A[Lab Test] --> B[Office test]; B --> C[End-user test];
```

Office test

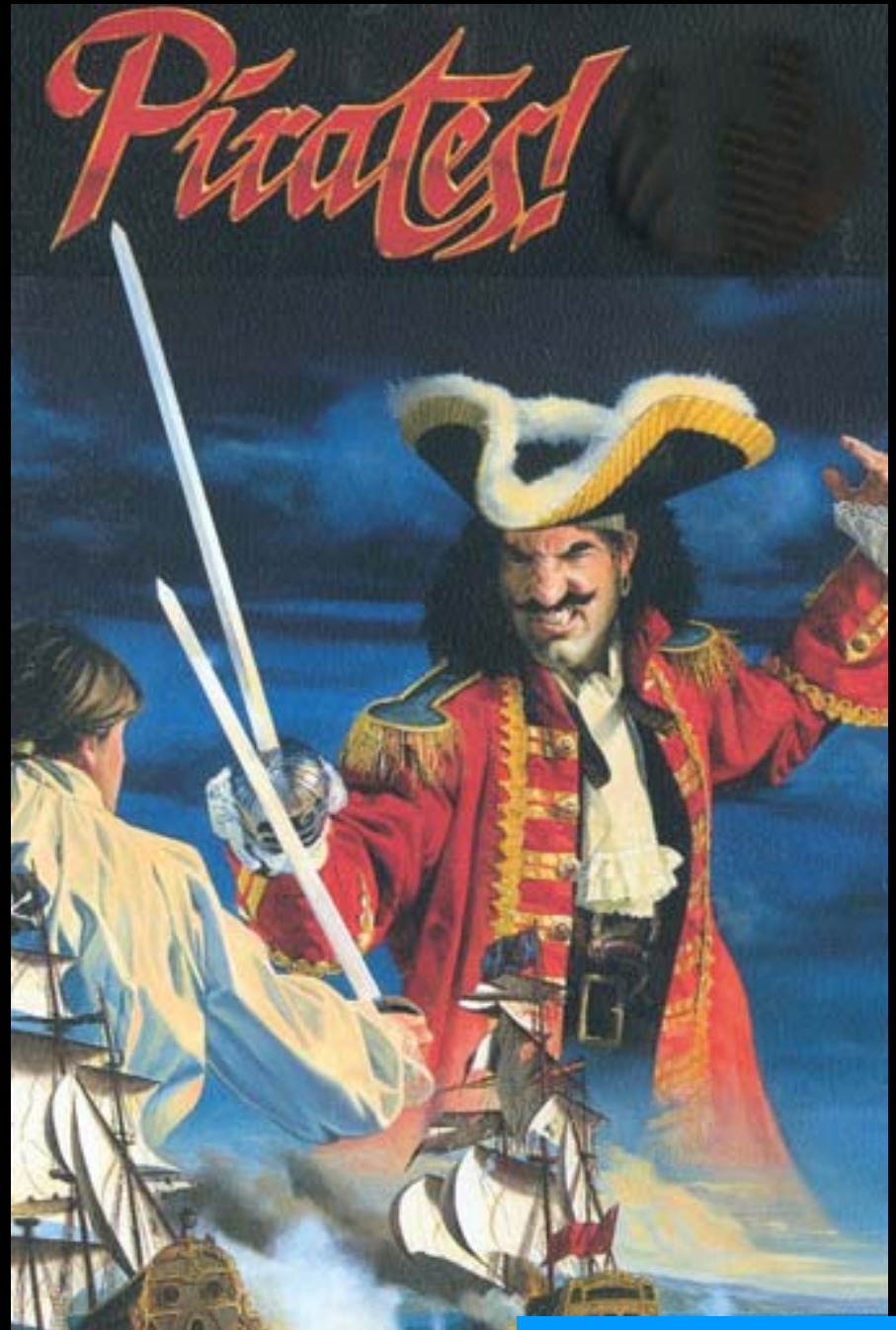
End-user test

Breathscanner 2.5

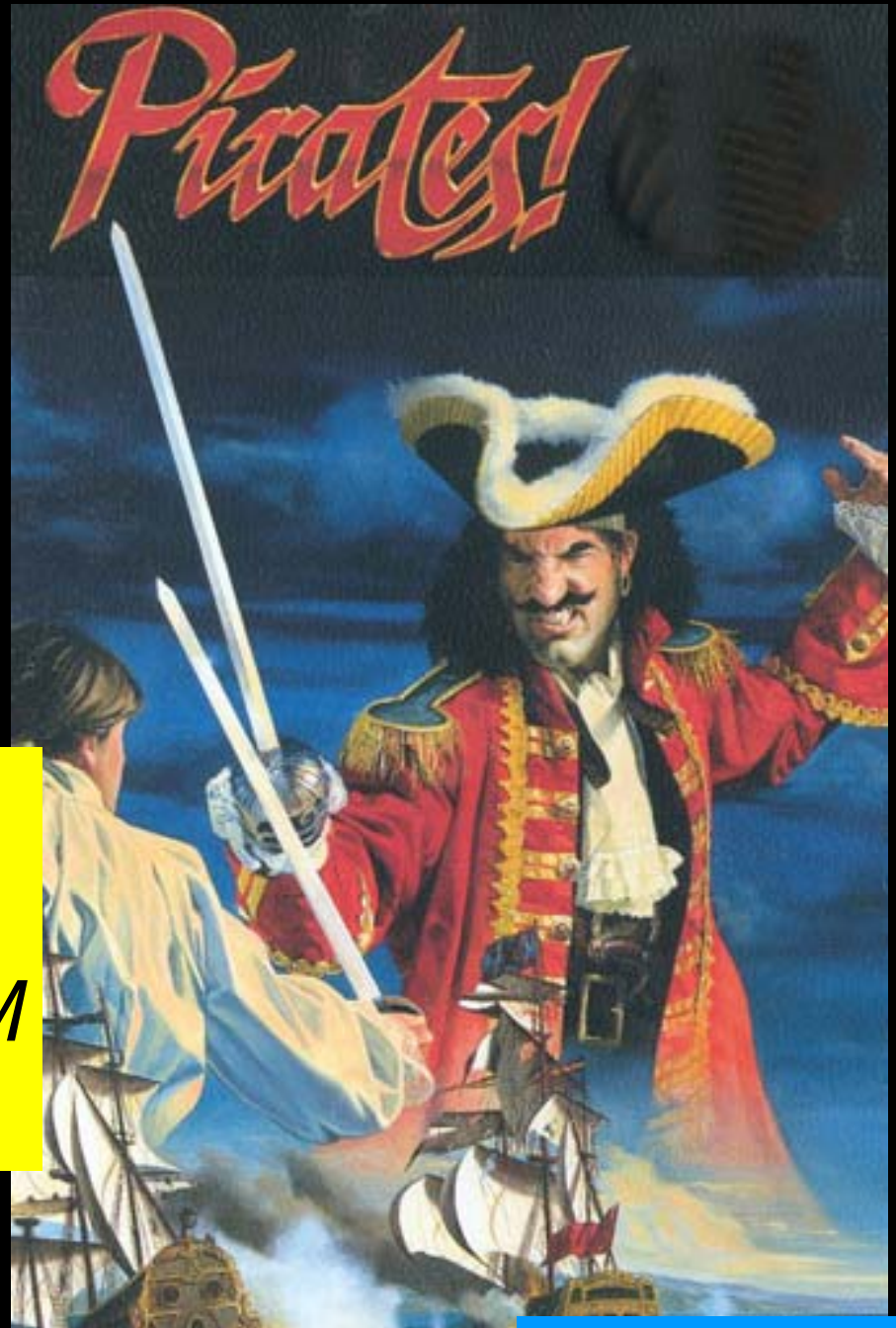
Point-of-care breath test
Runs on water and electricity
Results in minutes



*One more
problem....*

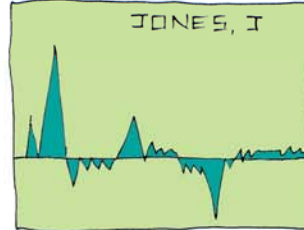


*PIRATES COULD
TRY TO STEAL
ALGORITHMS FROM
BREATHSCANNER*



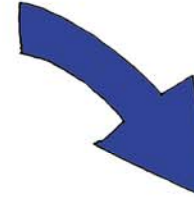
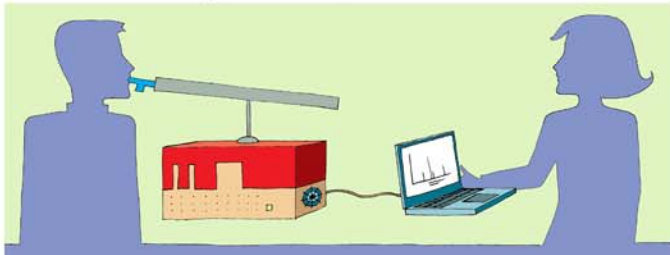
Remote diagnosis system

upload data



over internet

breath analysis anywhere in the world

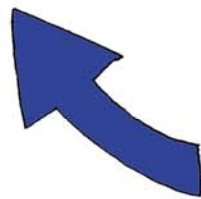


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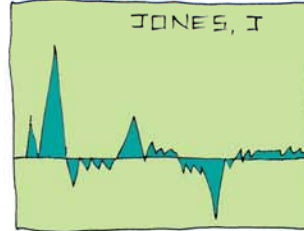


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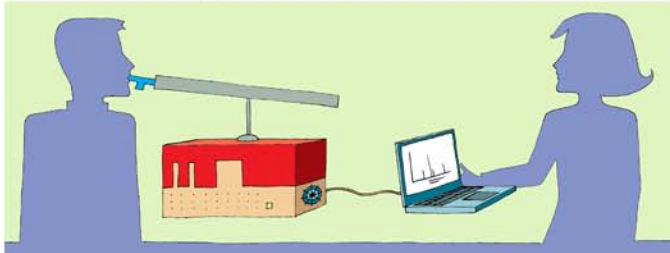
Remote diagnosis system

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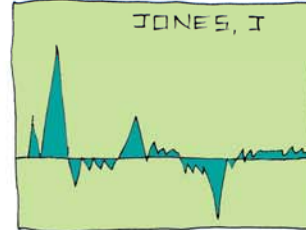


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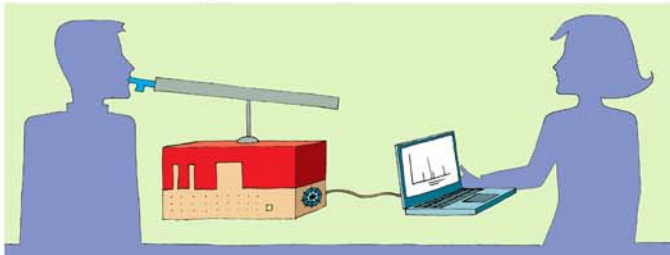
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